Amendments to the Specification

Please replace the Title with the following substitute Title:

COMPOSITIONS AND METHODS FOR PROMOTING ANGIOGENESIS

Please replace the paragraph beginning at page 21, line 3, with the following rewritten paragraph:

Proadrenomedullin N-terminal 20 peptide (PAMP): A 20 amino-acid molecule originating from the post-translational processing of pre-proadrenomedullin (see, for example, Ishimitsu *et al.*, *Biochem. Biophys. Res. Commun.* 203, 631-639, 1994). As described herein, the term PAMP includes the PAMP amino acid sequence shown in SEQ ID NO: 4, variant PAMP amino acid sequences that share 90% or 95% sequence identity with SEQ ID NO: 4 and retain angiogenic activity, PAMP fragments that retain angiogenic activity, and PAMP fusion proteins that retain angiogenic activity~~acivity~~. Angiogenic activity can be assessed using any of the angiogenesis assays described herein. As defined herein, a variant PAMP sequence, PAMP fragment, or PAMP fusion protein retains angiogenic activity if it retains at least a portion of the angiogenic activity of PAMP, for example 25%, 50%, 75%, 90%, or 95% of the angiogenic activity of PAMP, as measured in an angiogenesis assay, for example the DIVAA assay described herein.

Please replace the paragraph beginning at page 31, line 7, with the following rewritten paragraph:

The determination that an antibody inhibits the angiogenic activity of PAMP may be made, for example, using an angiogenesis assay, for instance any of the angiogenesis assays described herein (see section VII, below). For instance, the determination~~determination~~ that an antibody inhibits the angiogenic activity of PAMP can be made by comparing the angiogenic activity of PAMP alone with the angiogenic activity of PAMP in the presence of the PAMP antibody using the DIVAA assay. An antibody that inhibits the angiogenic activity of PAMP will reduce the angiogenic activity of PAMP in the DIVAA assay by a certain amount, for example, by 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even by 100%.

Please replace the paragraph beginning at page 54, line 14, with the following rewritten paragraph:

The chick embryo aortic arch assay is an *ex vivo* angiogenesis assay that was performed as previously described (Isaacs, *et al., J. Biol. Chem.,* 16;277(33):29936-44, 2002; Auerbach *et al., Clin.*

Chem. 49, 32-40, 2003). Briefly, aortic rings of approximately 0.8mm in length were prepared from the five aortic arches of 13 day-old chicken embryos (Truslow Farms) and the soft connective tissue of the adventitia layer was carefully removed with tweezers. Each aortic ring was placed in the center of a well in a 48-well plate and covered with 10 µl matrigel (BD Biosciences). After the matrigel solidified, 300 µl of growth factor-free human endothelial-SFM basal growth medium (~~Invitrogene~~Invitrogen) containing the proper concentration of the test substances were added to each well. The plates were kept in a humid incubator at 37 °C in 5% CO_2 for 24-36 hours. Microvessels sprouting from each aortic ring were photographed in an inverted microscope and the area covered by the newly formed capillaries was estimated by image analysis.

Please replace the paragraph beginning at page 55, line 2, with the following rewritten paragraph:

Analysis and quantitation of angiogenesis was done using DLVAA as previously described (Martinez *et al., J. Natl. Cancer Inst.,* 21;94(16):1226-37, 2002; Guédez *et al., Am.]. Pathol.* 162, 143 1-1439, 2003). Briefly, 10 mm long surgical-grade silicone tubes with only one end open (angioreactors) were filled with 20 µl of matrigel alone or mixed with AM, bFGF, VEGF, PAMP, and/or PAMP(12-20) at the indicated concentrations. Human lung cancer cell lines (see below) were also premixed with ~~matrigel~~matrigel alone or in combination with PAMP(12—20) at 10,000 cells per angioreactor. After the matrigel solidified, the angioreactors were implanted into the dorsal flanks of ~~anesthesized~~anesthetized athymic nude mice (NCI colony). After eleven days, the mice were injected intravenously with 25mg/ml FITC-dextran (100 µl/mouse, Sigma) 20 minutes before removing the angioreactors. Photographs of the implants were taken for visual examination of ~~angiosenic~~angiogenic response. Quantitation of neovascularization in the angioreactors was determined as the amount of fluorescence trapped in the implants and was measured in a HP Spectrophotometer (Perkin Elmer).

Please replace the paragraph beginning at page 55, line 16, with the following rewritten paragraph:

The human cancer cell lines used, A549 and H1299, were obtained from the American Tissue Culture Collection (ATTC) and fed with RPMI164O containing 10% fetal bovine serum (~~Invitrogene~~Invitrogen). Before they were used in animals, both cell lines were tested for a panel of human and murine pathogens and found to be pathogen-free.

Please replace the paragraph beginning at page 55, line 22, with the following rewritten paragraph:

Human dermal microvascular ~~endothelial~~endothelial cells were obtained from Cell Applications, Inc. and cultured in 96-well plates at 1.0 x 10^5 cells per well. The cells were loaded for 60 minutes at room temperature with the fluorescent dye FLIPR (Molecular Devices) and then transferred to the FlexStation II (Molecular Devices) for analysis. The test compounds were prepared in another plate at a concentration of 5x and were added to the proper wells by the robotic arm of the FlexStation II. Fluorescence was measured every five seconds in each well and recorded. One mM ATP (Sigma) was used as a calcium agonist (Lau *et al., Life Sci.* 73, 20 19-2028, 2003).

Please replace the paragraph beginning at page 58, line 25, with the following rewritten paragraph:

To further characterize this observation, an *in vitro* assay was employed that allows for more precise quantitation of angiogenic properties: the directed *in vitro* angiogenesis assay or DIVAA (Martinez *et al., J. Natl. Cancer Inst.,* 21;94(16):1226-37, 2002; Guédez *et al., Am.J. Pathol.* 162, 143 1-1439, 2003). The assay involves implanting small silicone capsules carrying the test substances under the skin of nude mice. After eleven days, the mice are injected with a specific amount of FITC-dextran, and the volume of blood circulating through the implant is quantified by measuring the fluorescence in the capsule. In addition, the new blood vessels growing into the silicone tube can be seen directly by transparency (FIG. 4A-F). Interestingly, PAMP was able to elicit an angiogenic response at concentrations as low as 1 femtomols/L (FIG. 4C). The extent of the angiogenic response can be seen clearly when this response is compared with the negative control (FIG. 4A). The angiogenic response elicited by PAMP was dose-dependent (FIG. 4C-G). When compared to and VEGF responses at equimolar concentrations, a clear ~~difference~~difference was observed. In this animal model, AM and VEGF began to induce angiogenesis at nanomolar concentrations, whereas PAMP was already active in the femtomolar range (FIG. 4G).

Please replace the paragraph beginning at page 59, line 12, with the following rewritten paragraph:

Although the AM receptor has been well characterized at the molecular level (McLatchie *et al., Nature* 393, 333-339, 1998), the structure of the PAMP receptor is not yet available. Nevertheless, exposure of adrenal medulla cells to PAMP results in a decrease of carbachol-induced calcium influx (Katoh *et al., J. Neurochem.* 64, 459-461, 1995). To create similar conditions in endothelial cells, cells were stimulated with 1 mM ATP, a well known transient agonist of ~~calcium~~calcium influx in these

cells (Lau *et al., Life Sci.* 73, 20 19-2028, 2003), obtaining a typical response (FIG. 5, squares). This response was greatly reduced by the presence of 10 nM PAMP in the medium (FIG. 5, diamonds). The peptide fragment PAMP(12-20) has been shown to have opposite actions to full-length PAMP in blood pressure regulation (Fry *et al., Life Sci.* 60, PL161-167, 1997), suggesting its potential utility as a PAMP antagonist. To demonstrate the specificity of the inhibition, an excess of the PAMP peptide fragment was added and the initial response was recovered (FIG. 5, circles). Taken together, these data show that there is a functional PAMP receptor in the membrane of the endothelial cells, and therefore this peptide may activate directly the angiogenic response described above.

Please replace the paragraph beginning at page 59, line 29, with the following rewritten paragraph:

For angiogenesis to occur, endothelial cells have to proliferate, migrate into new locations, and organize themselves into solid cords that eventually will develop into hollow tubes. All these processes are promoted by proangiogenic substances and all proangiogenic molecules must elicit at least one of these ~~physiologic~~physiological actions. To investigate which of these phenomena are induced by PAMP, human microvascular endothelial cells were exposed to increasing concentrations of PAMP, AM, and VEGF and their effects on growth were compared (FIG. 6A), migration (FIG. 6B), and cord formation (FIG. 6C).